JOURNAL OF THE WORLD AQUACULTURE SOCIETY

Vol. 25, No. 3 September, 1994

A Comparative Study on the Use of Different Preparations of Decapsulated Artemia Cysts as Food for Rearing African Catfish (Clarias gariepinus) Larvae

R. Pector

Laboratory for Ecology and Aquaculture, University of Louvain, Naamsestraat 59, B-3000 Leuven, Belgium

W. TACKAERT AND P. ABELIN

Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Ghent, Belgium

F. OLLEVIER

Laboratory for Ecology and Aquaculture, University of Louvain, Naamsestraat 59, B-3000 Leuven, Belgium

P. Sorgeloos

Laboratory of Aquaculture & Artemia Reference Center, University of Ghent, Rozier 44, B-9000 Ghent, Belgium

Abstract

African catfish (Clarias gariepinus) larvae were fed on five preparations of decapsulated Great Salt Lake Artemia cysts: 1) dried (35 C, 6 h) and UV-irradiated cysts; 2) heated cysts (80 C, 10 min); 3) brine-dehydrated and UV-irradiated cysts; 4) micro-bound diet 1 (with intact decapsulated cysts used in diet preparation 1); and 5) micro-bound diet 2 (with crushed and shifted decapsulated cysts used in diet preparation 1). The larvae were fed from the first day after yolk-sac resorption (fourth day after hatching). After a 14-d rearing period, larvae fed on dried or heated decapsulated cysts yielded significantly higher mean weights than the groups fed on brine-dehydrated decapsulated cysts or micro-bound diet 1. Feeding micro-bound diet 2 resulted in a significantly lower average gain in weight as compared to the other groups.

The importance of using freshly-hatched Artemia nauplii as a larval start feed for freshwater and marine fishes has often been emphasized (Léger et al. 1986; Sorgeloos et al. 1988). The nauplii, which can be produced from year-round commercially available cysts, apparently contain the essential macro- and micronutrients for the larvae. Moreover, Artemia nauplii provide a source of exogenous enzymes which may trigger or increase the proteolytic activity in the digestive tract of the larvae (Dabrowski 1979; Lauff and Hofer 1984).

The use of decapsulated cysts, rather than live nauplii, presents several advantages: e.g., separation of empty cyst shells is eliminated, decapsulated cysts are disinfected, and they have a higher dry weight and en-

ergy content (Vanhaecke et al. 1983). Dried decapsulated Artemia cysts may be used as an interesting alternative to live nauplii in larval nutrition studies. They do not leach, their particle size (just over 200 μ m) is appropriate for most fish larvae (Verreth et al. 1987), and most importantly they can be used as a non-living diet eliminating the inconvenience of producing live foods. Decapsulated cysts have been used as a standard reference diet for the larvae of Clarias gariepinus (Verreth et al. 1987).

Investigating the practical use of microbound diets (MBD) for red sea bream and Japanese flounder larvae, Kanazawa et al. (1989) concluded that substitution of artificial feeds for live food is possible. Yet, they stated that further improvements in the for-

mulation and preparation of MBD might still be necessary.

Prepared dry larval feeds reportedly encounter problems concerning particle size, attractivity, and losses of essential nutrients during preparation as well as through leaching in the water (Tacon and Cowey 1982; Dabrowski and Bardega 1984). This study investigated the nutritional value of different preparations of decapsulated Artemia cysts for rearing African catfish (Clarias gariepinus) larvae.

Materials and Methods

African catfish larvae were obtained by cross-breeding an African (Cameroon, Bangui) female with an Israeli (Hulagh swamps) male broodstock fish. Five duplicate groups of 300 larvae each (average weight on day 1: 2.29 mg) were transferred into 10 aquaria (40 L each) which were part of a recirculation system, including a sedimentation tank and a biological filter. The water temperature was maintained at 27.5 ± 1 C and the flow through each aquarium adjusted to 0.6 L/min.

Five experimental groups were fed on diets consisting of various preparations of decapsulated Great Salt Lake (GSL) Artemia cysts prepared as follows:

Treatment 1: UV-irradiated (30 Watt, 24 h, distance: 20 cm) and dried (35 C, 6 h) decapsulated cysts

Treatment 2: same as Treatment 1 but heated (80 C, 10 min) by immersing the cysts, packed in a plastic bag, for 10 min in water at 80 C

Treatment 3: UV-irradiated (30 Watt, 24 h, distance: 20 cm) and brine-dehydrated (saturated NaCl brine) decapsulated cysts Treatment 4: micro-bound diet (MBD) 1 prepared as follows: dried decapsulated cysts used in diet preparation 1 were mixed in water (heated to 80 C) and predissolved K-carrageenan (5 g/100 g dry feed). After cooling in a refrigerator, the paste was freeze-dried. The dried cake was then ground and sieved through a 260
µm mesh

Treatment 5: micro-bound diet (MBD) 2 prepared following the same procedure as for Treatment 4 but with crushed cysts (particle size $\leq 100 \ \mu m$)

Feeding of larvae was started the day after yolk-sac resorption (day 1). Prior to administration the diets were moistened for a few seconds in freshwater to facilitate ingestion by the larvae. The expected daily growth was determined by the linear relation existing between the length of the rearing period and the average body weight of the larvae (Hogendoorn 1980). Daily feed requirements (on a dry weight basis) for each group were calculated on the basis of the expected growth and a predicted food conversion rate of 3. All diets were administered manually four times a day between 0900 h and 1800 h. Quantities given were assumed to be the actual amount consumed by the larvae.

On days 4, 6, 11 and 14 the mean weight and length were determined from 20 randomly sampled and anesthetized larvae from each tank. Cleaning of aquaria and counting of dead larvae were done daily.

The percentage of cannibalism was calculated as follows:

% cannibalism = 100% - % mortality
- % survival.

The feed conversion rate (FCR) was calculated as the total quantity of feed supplied (dry weight)/total gain of fish biomass (wet weight). Water stability of the feeds was calculated as the percentage of dry matter remaining after 10 min and after 1-h immersion of the feeds in rotating tubes filled with freshwater, centrifuged for 20 min and the supernatant discarded.

When the analysis of variance demonstrated that significant differences were present among treatments, Duncan's multiple range test (Steel and Torrie 1960) was used to determine differences in mean weight and length between the groups. All statistical analyses were performed using SAS General Linear Models Procedure (SAS In-

Table 1. Mean weights (mg) of Clarias gariepinus larvae fed different preparations of decapsulated Artemia cysts. Weights in the same column with the same superscript are not significantly different (P < 0.05). The mean weight on day 1 was 2.29 \pm 0.35 mg. No differences in mean weight among both duplicate aquaria of each experimental group were noted.

Treatment	Mean weight (mg) (N = 40)				
	Day 4	Day 6	Day 11	Day 14	
1	9.11 ± 0.95^{a}	17.62 ± 3.12^{a}	49.85 ± 10.34 ^a	83.00 ± 16.89 ^a	
2	8.34 ± 1.15^{b}	15.95 ± 3.45^{b}	47.23 ± 9.49^a	79.46 ± 14.98^{a}	
3	9.41 ± 1.57^{a}	17.39 ± 3.62^a	39.24 ± 7.03^{b}	64.58 ± 10.62 ^b	
4	$5.58 \pm 0.84^{\circ}$	11.00 ± 2.43^{c}	39.52 ± 13.05^{b}	64.74 ± 16.52^{t}	
5	4.38 ± 1.04^{d}	3.75 ± 1.09^{d}	$6.58 \pm 1.73^{\circ}$	$6.77 \pm 2.46^{\circ}$	

stitute Inc. 1985). All differences were considered significant at the P < 0.05 level.

Results

At day 4 the mean weight of the larvae (Table 1) was highest in Treatments 1 and 3, whereas the mean length was highest in treatment 3 (Table 2). The average weight obtained with the heated decapsulated cysts was higher than with the MBD 1 (prepared with the intact cysts). The larvae receiving the MBD 2 (prepared with the crushed and sifted cysts) showed the lowest mean weight.

After 14 d of culture, the larvae fed on dried or heated decapsulated cysts (Treatments 1 and 2) yielded the highest mean weights (Table 1) and lengths (Table 2), followed by Treatments 3 and 4. On the other hand, the MBD 2 resulted in the lowest mean weight (Table 1) and mean length (Table 2).

The best food conversion rate was obtained in larvae fed on brine dehydrated cysts (Treatment 3) (Table 3). Similar food conversion rates were obtained among lar-

vae fed on dried or heated decapsulated cysts and the MBD 1. The larvae fed on the MBD 2 had the poorest food conversion rate.

After 14 d of culture, high survival rates (Table 3) were obtained in all experimental groups. A relatively lower survival rate was obtained for the larvae fed on the MBD 2. Compared to the other groups the percentage of cannibalism was also higher for this group.

Analysis of the water stability of the diets (Table 4) indicates that dried decapsulated cysts are very water stable. Heated and brine dehydrated cysts have a lower, but still acceptable, water stability. In MBD 1 and especially MBD 2 the % dry matter retention after 10 min immersion in water is, however, considerably reduced.

Discussion and Conclusions

The use of decapsulated cysts as a direct food source eliminates the need for hatching the cysts and implies several other advantages (Léger et al. 1986):

Table 2. Mean lengths (mm) of Clarias gariepinus larvae, fed different preparations of decapsulated Artemia cysts. Lengths in the same column with the same superscript are not significantly different (P < 0.05). No differences in mean length among both duplicate aquaria of each experimental group were noted.

	Mean length (mm) $(N = 40)$				
Treatment -	Day 4	Day 6	Day 11	Day 14	
1	9.90 ± 0.65 ^b	12.65 ± 1.04^a	18.15 ± 1.15 ^a	20.34 ± 1.87 ^a	
2	9.67 ± 0.80^{b}	12.28 ± 1.04^{ab}	17.52 ± 1.25^{b}	20.11 ± 1.50^{a}	
3	10.68 ± 0.53^{a}	11.91 ± 0.83^{ab}	16.94 ± 1.10^{c}	18.78 ± 1.34^{t}	
4	8.07 ± 0.47°	$10.41 \pm 0.59^{\circ}$	16.55 ± 1.67^{c}	18.45 ± 1.82 ^b	
5	7.45 ± 0.82^{d}	6.72 ± 0.97^{d}	9.04 ± 1.42^{d}	$8.10 \pm 1.04^{\circ}$	

Table 3. Food conversion rate (FCR) and the percentages of survival, mortality and cannibalism of Clarias gariepinus larvae fed with different preparations of decapsulated Artemia cysts.

	-			
Treat- ment	FCR	% surival	% mortality	% cannibalism
1	1.2	98	1	1
2	1.2	98	1	1
3	0.8	98	1	1
4	1.2	97	2	1
5	1.8	76	14	10

- decapsulated cysts are sterile thus eliminating the potential risk of introducing germs via hatched nauplii into the rearing system.
- because their diameter and volume are smaller (30-40%) than in freshly-hatched nauplii (Vanhaecke 1983) they can be fed to earlier larval stages.
- 3) the energy content of decapsulated cysts is 30-57% higher than in freshly-hatched nauplii (Vanhaecke et al. 1983).
- cysts that have lost the capacity to hatch may still be used as a food source in aquaculture.

The main problem when using decapsulated cysts as a direct food source is their fast sedimentation in water, which makes them unavailable for planktonic larvae unless they hatch. The use of dried decapsulated cysts which float and upon hydration sink only slowly may provide a good alternative.

In this experiment it is shown that brine-dehydrated decapsulated cysts are an acceptable starter feed for *Clarias gariepinus* larvae, producing the highest mean weight after only 4 d of rearing. A positive effect on the osmoregulatory system of these very young larvae due to the presence of ions such as Na⁺, Cl⁻ in brine can be a cause of improvement for gain in body weight. However, Van Damme et al. (1990) obtained a higher mean weight (16.26 mg) at day 4 when feeding *Artemia* nauplii to African catfish larvae.

In the period between days 6 and 11, how-

TABLE 4. Water stability of the diets.

	Water stability of the diets (% of dry matter remaining after immersion in freshwater)		
Diet	aster 10 min	after 1 h	
1	92.42	90.02	
2	87.94	78.21	
3	84.20	76.32	
4	75.52	72.52	
5	71.46	64.68	

ever, the beneficial effect of the brine seems to disappear (Table 1). On day 11 the mean weight of the larvae fed on the brine-dehydrated cysts has indeed become significantly lower than the mean weight of the larvae fed on dried or heated decapsulated cysts. A better knowledge of the ontogenic development and functioning of the osmotic regulating system of the *C. gariepinus* larvae could provide more information to explain these results.

The differences in growth after a 14-d culture can possibly be related to the water stability of the diets (Table 4); i.e., the better the water stability of the diet, the higher the average weight gained. Especially in the MBD 2, poor water stability due to destruction of the outer cuticular membrane may have caused a considerable loss of essential nutrients through leaching in the water, resulting in an overall poor performance of this diet. Also the smaller (well below the optimum) particle size of the MBD 2 (29.6% $\geq 200 \,\mu\text{m}$; 58.6% between 100–200 μm and $11.8\% \le 100 \,\mu\text{m}$) as compared to the other diets (235-260 μ m) may have further contributed to the poor growth results of the larvae fed on this diet. Uys (1984) reported that the optimum particle size for Clarias gariepinus larval diets is 2.2% of the mean length of the fish.

Heating the cysts to 80 C did not produce a significant effect on growth and survival of the larvae after 14 d of culture. This could indicate that exposure of decapsulated cysts to temperatures of 80 C does not induce biochemical changes, such as loss of en370 PECTOR ET AL.

zymes or growth factors, leading to a reduced nutritional value of the cysts for *Clarias gariepinus* larvae. A similar phenomenon was reported by Hinton (1968) who found that cysts heated to 80 C still retained their capacity to hatch.

In conclusion, the results of the present study demonstrate that brine-dehydrated decapsulated *Artemia* cysts are an acceptable start feed for African catfish larvae.

Feeding with decapsulated Artemia cysts for more than 4 d is, however, not recommended since the size of this food organism is suboptimal for C. gariepinus juveniles (Van Damme et al. 1990).

It is also shown that, for the preparation of micro-bound diets, special attention should be paid to the selection of the appropriate particle size (in function of the size of the larvae) and the water stability of the diets.

Literature Cited

- Dabrowski, K. 1979. The role of proteolytic enzymes in fish digestion—a new approach. In E. Styczynska-Jurewicz, T. Backiel, E. Jaspers and G. Persoone, editors. Cultivation of fish fry and its life food. Special Publication of the European Mariculture Society 4:107-126.
- Dabrowski, K. and R. Bardega. 1984. Mouth size and predicted food size preferences of larvae of three cyprinid fish species. Aquaculture 40:41-46.
- Hinton, H. E. 1968. Reversible suppression of metabolism and the origin of life. Proceedings of the Royal Society, series B 171:43-56.
- Hogendoorn, H. 1980. Controlled propagation of the African catfish, Clarias lazera (C. & V.). III. Feeding and growth of fry. Aquaculture 21:233-241.
- Kanazawa, A., S. Koshio and S. Teshima. 1989. Growth and survival of larval red sea bream Pagrus major and Japanese flounder Paralichthys olivaceus fed microbound diets. Journal of the World Aquaculture Society 20(2):31-37.
- Lauff, M. and R. Hofer. 1984. Proteolytic enzymes in fish development and the importance of dietary enzymes. Aquaculture 37:335-346.

- Léger, P., D. A. Bengtson, K. L. Simpson and P. Sorgeloos. 1986. The use and nutritional value of Artemia as a food source. Oceanography and Marine Biology: An Annual Review 24:521-623.
- SAS Institute Inc. 1985. SAS user's guide: statistics, 5th edition. SAS Institute Incorporated, Cary, North Carolina, USA.
- Sorgeloos, P., P. Léger and P. Lavens. 1988. Improved larval rearing of European and Asian seabass, seabream, mahi-mahi, siganid and milkfish using enrichment diets for *Brachionus* and *Artemia*. World Aquaculture 19(4):78-79.
- Steel, R. G. D. and J. H. Torrie, editors. 1960. Principles and procedures of statistics. McGraw-Hill Book Company, New York, New York, USA.
- Tacon, A. G. J. and C. B. Cowey. 1982. Fish nutrition. Relevance to invertebrates. Pages 13-30 in E. D. Pruder, C. J. Langdon and D. E. Conklin, editors. Proceedings 2nd International Conference on Aquaculture Nutrition. WMS Special Publication no. 2. Louisiana State University Press, Baton Rouge, Louisiana, USA.
- Uys, W. 1984. Investigations into the dietary requirements of Clarias gariepinus larvae (Pisces: Claridae) and the formulation and manufacture of an artificial dry feed for use in intensive larval rearing. Master's thesis. Rhodes University, Grahamstown, South Africa.
- Van Damme, P., H. Rutten and F. Ollevier. 1990. The adaptation of Clarias gariepinus larvae to dry feed. Pages 303-306 in R. Hirano and I. Hanyu, editors. 1990. The Second Asian Fisheries Forum. Asian Fisheries Society, Manila, Philippines.
- Vanhaecke, P. 1983. Vergelijkende studie van diverse geografische rassen van het pekelkreeftje, Artemia, ter verbetering van zijn gebruik in de aquacultuur. Doctoral thesis. State University of Ghent, Belgium.
- Vanhaecke, P., P. Lavens and P. Sorgeloos. 1983. International study on Artemia. XVII. Energy consumption in cysts and early larval stages of various geographical strains of Artemia. Annales de la Société royale zoologique de Belgique 113(2):155-164.
- Verreth, J., V. Storch and H. Segner. 1987. A comparative study on the nutritional quality of decapsulated Artemia cysts, micro-encapsulated egg diets and enriched dry feeds for Clarias gariepinus (Burchell) larvae. Aquaculture 63:269-282.